

AWARD NUMBER: W81XWH-14-1-0359

TITLE: Targeting ESR1-Mutant Breast Cancer

PRINCIPAL INVESTIGATOR: Dr. Sarat Chandarlapaty

CONTRACTING ORGANIZATION: Sloan Kettering Institute for Cancer Research
New York, NY 10065

REPORT DATE: September 2015

TYPE OF REPORT: Annual Technical Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE September 2015		2. REPORT TYPE Annual Progress Report		3. DATES COVERED 1 Sep 2014 - 31 Aug 2015	
4. TITLE AND SUBTITLE Targeting ESR1-Mutant Breast Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-14-1-0359	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Sarat Chandarlapaty E-Mail: chandars@mskcc.org				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) SLOAN_KETTERING INSTITUTE FOR CANCER RESEARCH 1275 YORK AVE New York, NY 10065-6007				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The hypothesis of this proposal is that LBD mutations in ESR1 promote resistance to current FDA approved hormonal therapies and that more potent, selective estrogen receptor degraders (SERDs) will enable complete inhibition of mutant ER signaling and thus have substantial therapeutic benefit. Our first aim in this proposal was to determine the biochemical and biologic impact of ER mutations found in breast cancer using both structural and cell based assays. We have now have evidence for the effects of the most recurrent mutations, D538G and Y537S inducing an agonist apo-ER structure and promoting estrogen independent tumor growth as well as preliminary evidence for the mutants promoting transcriptional effects including but also beyond those induced by estrogen. Additional aims were to understand how mutant ER is impacted by SERD compounds again using structural, cell-based, and mouse models as readouts. We have evidence that the Y537S and D538G alterations modify the SERM (4OHT) induced antagonist structure to a greater extent than SERDs (bazedoxifene), suggesting SERDs as potentially superior agents. Our biologic data from a suite of ER antagonists suggest the same, that SERDs such as bazedoxifene or GDC0180 potentially inhibit wild type and mutant ER driven phenotypes. Overall, the work is suggesting rational steps forward towards targeting ER mutant driven cancers.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	13	19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	5
3. Accomplishments.....	6-8
4. Impact.....	9
5. Changes/Problems.....	10
6. Products.....	11
7. Participants & Other Collaborating Organizations.....	12
8. Special Reporting Requirements.....	13
9. Appendices.....	13

1. Introduction

Approximately 70% of ER+ breast cancers harbor expression of the estrogen receptor and are dependent upon its activity for various aspects of the transformed phenotype. Lethal outcome from breast cancer almost invariably comes due to metastatic progression of disease. Much like with primary breast cancer, approximately 70% of metastatic breast cancer displays ER expression. However, the targeting of the estrogen receptor in this setting is not curative and is associated with acquired resistance to antiestrogen therapy. We have found that mutation in the estrogen receptor is highly prevalent in such ER+ metastatic breast cancers. The mutations occur in the ligand binding domain and our preliminary data is that the mutations promote receptor function in the absence of ligand. The hypothesis of this proposal is that mutations in ESR1 ligand binding domain will also promote resistance to current FDA approved ER antagonists, but that more potent and selective estrogen receptor antagonists will be sufficiently active to target mutant receptors and offer substantial therapeutic benefit in the clinic. The proposal aims to combine structural, biochemical, and pharmacologic analyses of the most common ER mutants to identify drugs that are most rational to move forward in the clinic.

2. Keywords

Estrogen Receptor

Estrogen Response Element

Metastatic Breast Cancer

Ligand Binding Domain

Mutation

Antagonist

Selective Estrogen Receptor Modulator

Selective Estrogen Receptor Degradar

Tamoxifen

Fulvestrant

Bazedoxifene

Raloxifene

GDC-0810

AZD9496

3. Accomplishments

A) Major goals and progress towards each goal.

1) Characterize effects of mutants on ER signaling and structure

The hypothesis for this section was the ER mutants would drive ER driven gene expression profiles, alter ER structure and alter ER affinity to ligands. The work on ER affinity and structure will be reported by Site#2. For Site 1, it was anticipated that it would take 36 months to complete expression profiling and analyses. During Year#1, we have developed models of mutant ER driven cancer in which to characterize gene expression. Specifically, tetracycline inducible MCF7 cells that can drive D538G and Y537S have been generated along with control models that can induce expression of wild type or vector. These have included careful characterization of the doses of doxycycline that induce comparable levels of receptors under these conditions (Figure 1). This is necessary to help ensure changes in gene expression are not simply due to differences in ER level which may be from the

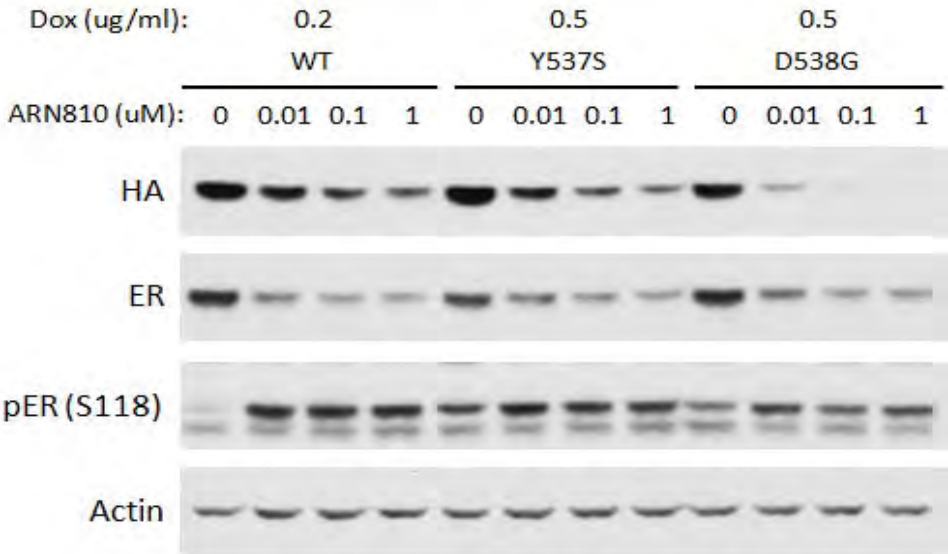


Figure 1: Degradation of WT and mutant ER with ARN810. MCF7 cells stably transfected with tet-inducible WT and mutant ER are then treated for 4 hours with ARN810 at indicated concentrations and immunoblotted.

Using these doses we have characterized gene expression profiles of the models and found significant overlap between the effects of wild type plus estradiol, and Y537S without estradiol and D538G without estradiol (data not shown). However, to our surprise, we have identified a significant partition of genes that are specific to the Y537S and D538G expressing cells when all are given in the presence of estradiol (Figure 2). To further validate this, we are attempting to develop Crispr knock-in models of the mutants in which to conduct gene expression profiling.

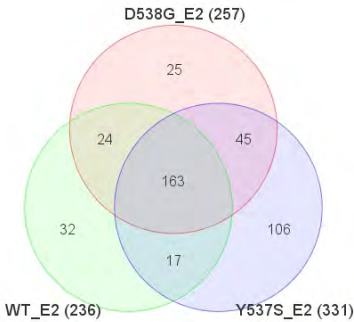


Figure 2: Gene expression profiling of WT and mutant ER. MCF7 cells stably transfected with tet-inducible WT and mutant ER are treated for 24 hours with estradiol and gene expression profiling performed with genes altered (1.5x) compared with WT without E2 shown. 163 genes were shared among the three cell lines but many were unique to Y537S (106), D538G (25), or both (45).

2) Characterize the oncogenic phenotypes of ER mutants

The major goal of this section is to determine the effect of the common ER mutants on ability to promote cell proliferation in hormone (estrogen) deprived conditions. Essential to this was the development of models which was accomplished as noted above. The ability of different mutants to sustain proliferation in hormone deprived media is shown below in Figure 3. Moreover, the ability of the mutants to promote xenograft tumor growth in the absence of estradiol has been further demonstrated by our group. These are key findings for the field and demonstrate the sufficiency of these mutants to elaborate an essential hallmark of hormone dependent cancer.

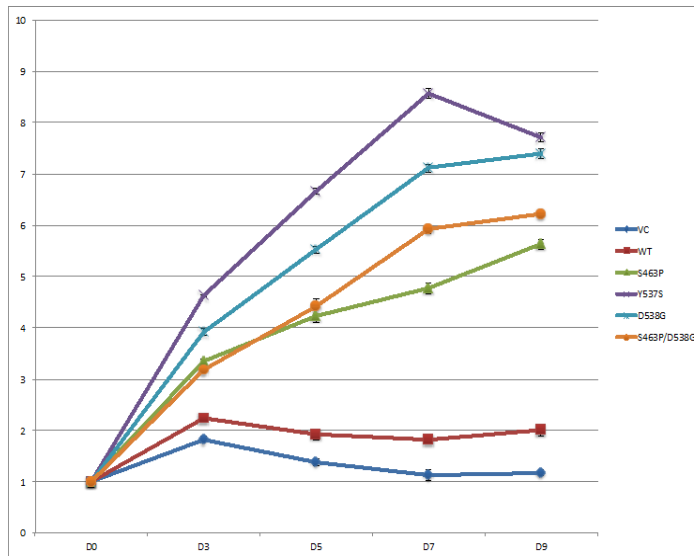


Figure 3: ER LBD mutants promote hormone independent proliferation. MCF7 cells stably transfected with tet-inducible WT (red) and mutant ER (S463P (green), Y537S (purple), D538G (blue), and S463P/D538G (orange)) or vector (navy) are grown in absence of estradiol beginning at day-2 and evaluated for proliferation at day 0, 3, 5, 7, and 9. Cells are administered doxycycline on Day 0 at doses known to induce similar levels of transgene.

3) Analyze effects of SERDs on ER biochemical activities

Major goals here are to evaluate effects on mutants on gene expression and then how SERDs alter growth. These were planned predominantly for year#2 of the grant period and have now been initiated.

4) Analyze effects of SERDs on ER structures

These are relevant to the Site#2 work and progress will be reported by Dr. Greene.

5) Efficacy of single agent SERD on mutants driven signaling in vivo

Major goal here is to establish the ability of individual and promising SERDs to antagonize mutant driven signaling in vivo using xenograft models. Plan to analyze the effects would take place over months 1-30 of the grant. We have initiated these studies using the tet-inducible models and shown the mutants to grow as xenografts and promote estrogen independent growth. We have now initiated studies to examine the effects of the AZD9496 and GDC0810 compounds on ER dependent signals. The work is proceeding well but no conclusion can be drawn right now.

6) Efficacy of SERD compounds on mutant driven tumor phenotypes

Major goal here is to establish the ability of individual and promising SERDs to antagonize mutant driven phenotypes in vivo using xenograft models. This was predominantly planned for Year# 3 of the grant period and we are well on track to accomplish these studies.

B) Opportunities for training and professional development

Training and professional development was not an explicit goal of this project.

C) Dissemination of results to community

Partial results of the project were reported in oral presentations given by the PI at the AACR Annual meeting in Philadelphia, PA and the Gordon Conference in Newry, Maine. An initial set of publications is currently being drafted, one by Site#1 and two by Site#2. We hope these will be published by the time of next year's progress reporting.

D) Plans for upcoming reporting period

The project plans for Year#2 are in line with our initial Statement of Work. For Year#2, we will aim to characterize the gene expression signatures unique to mutant ER in the hopes of further finding ER mutant specific functions and signatures. We will continue to characterize the ability of different ER antagonists and their potency against ER mutants both in vitro and in vivo.

4. Impact

A) Impact on development of the principal discipline

The major impact to date is the elucidation of how the genetic mutation in ER that is observed in 20-30% of hormone resistant breast cancer function on ER signaling, ER conformation, and antagonist response. We have established that the most common ER mutants possess sufficient intrinsic activity to cause hormone independent growth. We explain this by demonstrating the structure that the mutants cause the receptor to adopt mimics the estrogen bound structure. This explains the biologic selection for these alterations during treatment with drugs such as aromatase inhibitors that ablate estrogen production. We have made major strides in characterizing the proficiency of ER antagonists to target these mutants. While this part of the work is ongoing, the impact of understanding how best to target these alterations is expected to be profound going straight to clinical testing of these antagonists to replace existing ones that are ineffective against the mutants.

B) Impact on other disciplines

Nothing to report.

C) Impact on technology transfer

Nothing to report.

D) Impact on society beyond science

Nothing to report.

5. Changes

Nothing to report.

6. Products

A) Journal publications

Site#2 has a manuscript under review. Please see separate report from their site regarding this publication.

B) Presentations

- 1) AACR Annual Meeting. Invited oral presentation.
- 2) Gordon Research Conference. Invited oral presentation.

C) Other products

Nothing to report.

7. Participants

Name: Sarat Chandarlapaty

Project role: PI

Researcher Identifier:

Nearest person work month: 2

Contribution to project: Dr. Chandarlapaty has supervised the laboratory work conducted on this project include study design, experimental interpretation, and reporting.

Funding support: NIH, Damon Runyon Foundation, Komen Foundation

Name: Weiyi Toy

Project role: Postdoctoral Fellow

Researcher Identifier:

Nearest person work month: 6

Contribution to project: Dr. Toy has helped to conduct the laboratory work on this project include study design, experimental interpretation, and reporting.

Funding support: Philanthropic sources

Name: Zhiqiang Li

Project role: Postdoctoral Fellow

Researcher Identifier:

Nearest person work month: 6

Contribution to project: Dr. Li has helped to conduct the laboratory work on this project include study design, experimental interpretation, and reporting.

Funding support: Philanthropic sources, Damon Runyon Cancer Research Foundation

8. Special Reporting Requirements

Please see separate report from Site#2, Partnering PI – Dr. Geoffrey Greene.

9. Appendices

Nothing to report